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## Absence of corn stunt spiroplasma and maize bushy stunt phytoplasma in leafhoppers (Hemiptera: Cicadellidae) that inhabit edge grasses throughout winter in Jalisco, Mexico

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Field margins (edges) in certain crops are important because they form habitats that maintain herbivorous insects and their parasitoids and predators (Ramsden et al. 2015). However, little is known about whether these herbivores carry plant pathogens, such as bacteria that can infest the crops. In Mexico, 97% of maize (*Zea mays* L. ssp. *mays*; Poales: Poaceae) is planted annually during the maize-growing wet season (Jun to Oct) (Moya-Raygoza et al. 2004). Once the maize dries out, green grasses that grow on the edges of the maize fields serve as food resources for the overwintering insects. These monocots are colonized by herbivorous leafhoppers (Hemiptera: Cicadellidae) capable of harboring viral and bacterial pathogens of maize plants, leading to concerns that they could transmit these pathogens to crops during the next maize-growing wet season (Moya-Raygoza et al. 2007). The bacterial pathogens corn stunt spiroplasma (CSS), *Spiroplasma kunkelii* Whitcomb et al. (Entomoplasmatales: Spiroplasmataceae), and maize bushy stunt phytoplasma (MBSP), '*Candidatus* Phytoplasma asteris' (Acholeplasmatales: Acholeplasmataceae), are transmitted efficiently by the leafhoppers *Dalbulus maidis* Delong and *D. elimatus* Ball, both of which are important pests of maize in Latin America (Nault 1990). Furthermore, other leafhoppers such as *Graminella nigrifrons* Forbes, *Stirellus bicolor* Van Duzee, and *Exitianus exitiosus* (Uhler) are also vectors of CSS and/or MBSP (Nault 1980). The objective of this study was therefore to determine whether or not leafhoppers that inhabit edge grasses carry either of the pathogens (CSS or MBSP).

The study was conducted in the region of Zapopan, state of Jalisco, Mexico (20°44'N, 103°30'W; 1,662 m). Sampling was conducted on fallow perennial grasses of the family Poaceae; *Brachiaria brizantha* (A. Richard) Stapf, *Chloris gayana* Kunth, *Cynodon dactylon* (L.) Persoon, *Melinis minutiflora* P. Beauvois, *Panicum maximum* Jacquin, and *Rhynchelytrum repens* (Willdenow). All grasses showed green foliage during the sampling period. Leafhopper adults were collected from the grasses, using a sweep net, at least once a week during the winter (dry season), from Feb to Jun 2014. Sweep net collections of leafhoppers were conducted for an hour between 11 a.m. and 12 p.m., performing

1,800 sweeps on each sampling date. The collected leafhoppers were maintained in 95% ethanol for future identification and DNA extraction. Leafhoppers were identified to genus or species level using keys and previously identified leafhoppers for comparison. Voucher specimens were deposited in the entomological collections of the University of Guadalajara, Jalisco, Mexico.

DNA was extracted from each individual leafhopper by using the protocol developed by Aljanabi & Martinez (1997). CSS was detected by polymerase chain reaction (PCR) amplification of the CSS *spiralin* gene, following the method of Barros et al. (2001). Previously extracted CSS DNA was included in each gel as a positive control. MBSP was detected by PCR amplification of the phytoplasma *16S rRNA* gene from the leafhopper DNA extracts. First, we used the universal primer pairs P1 (Deng & Hiruki 1991) and Tint (Smart et al. 1996) using the PCR conditions described by Smart et al. (1996) to obtain preliminary results. We also used the leafhopper DNA extracts as the template for a nested PCR assay. Universal primer pairs P1 (Deng & Hiruki 1991) and P7 (Schneider et al. 1995) were used for the first reaction, and R16F2n/R16R2 (Gundersen & Lee 1996) for the nested reaction. In both cases, besides the leafhopper DNA extract, we used DNA extract from macadamia trees affected by '*Candidatus* Phytoplasma asteris' (Pérez-López et al. 2013) as positive controls. The PCR products were run on a 1.0% agarose gel for 1 h at 80 V and stained with ethidium bromide to visualize the positive bands for CSS (600 bp) and MBSP (1,600 bp and 1,250 bp for the P1/Tint and nested PCR reactions, respectively).

In total, we collected 2,263 leafhopper individuals belonging to 27 taxa. The most abundant species were *S. bicolor* and *Graminella sonora* (Ball) (Table 1). All collected taxa were negative for CSS and MBSP during the winter. This suggests that leafhoppers that inhabit edge perennial grasses, at least in the sampled area during winter, do not harbor CSS or MBSP.

CSS and MBSP are transmitted by *D. maidis*, *D. elimatus*, and *G. nigrifrons*, whereas CSS, but not MBSP, is transmitted by *S. bicolor*, *E. exitiosus* (Nault 1980), and *Exitianus obscurinervis* Stål (Carloni et al.

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**Table 1.** Abundance of leafhoppers species on edge grasses throughout winter 2014 in Jalisco, Mexico.

Taxon	Feb	Mar	Apr	May	Jun	Total
<i>Acinopterus</i> sp.	1 (1)	2 (2)	2 (2)	0	0	5 (5)
<i>Acuera</i> sp.	0	1 (1)	3 (2)	5 (3)	0	9 (6)
<i>Agallia</i> sp.	4 (2)	2 (2)	0	0	0	6 (4)
<i>Amblycellus</i> sp.	10 (2)	18 (5)	55 (4)	195 (5)	0	278 (16)
<i>Apogonalia</i> sp.	0	0	0	10 (2)	0	10 (2)
<i>Balclutha</i> sp.	26 (10)	107 (12)	24 (5)	3 (2)	0	160 (29)
<i>Dalbulus elimatus</i> *, **	2 (1)	0	0	0	0	2 (1)
<i>Dalbulus maidis</i> *, **	1 (1)	1 (1)	0	0	0	2 (2)
<i>Draeculacephala</i> sp.	17 (3)	7 (4)	5 (2)	0	0	29 (9)
<i>Empoasca</i> sp.	1 (1)	0	0	0	1 (1)	2 (2)
<i>Exitianus excavatus</i>	2 (1)	9 (3)	2 (2)	1 (1)	0	14 (7)
<i>Exitianus picatus</i>	21 (8)	39 (9)	24 (8)	30 (8)	0	114 (33)
<i>Graminella comata</i>	3 (2)	5 (4)	0	0	0	8 (6)
<i>Graminella sonora</i>	54 (16)	184 (23)	195 (9)	311 (11)	2 (1)	746 (60)
<i>Juitepeca</i> sp.	1 (1)	0	0	0	0	1 (1)
<i>Ollarianus</i> sp.	1 (1)	1 (1)	0	0	0	2 (2)
<i>Paraphlepsius</i> sp.	3 (2)	3 (3)	2 (1)	0	0	8 (6)
<i>Planicephalus lavicosta</i>	17 (3)	10 (4)	4 (3)	4 (2)	0	35 (12)
<i>Plesiommata</i> sp.	0	1 (1)	1 (1)	2 (1)	0	4 (3)
<i>Polyamia</i> sp.	1 (1)	3 (2)	3 (1)	3 (1)	0	10 (5)
<i>Stirellus</i> sp.	0	0	2 (1)	0	0	2 (1)
<i>Stirellus bicolor</i> *	50 (14)	145 (28)	197 (22)	398 (19)	4 (2)	794 (85)
<i>Sibovia</i> sp.	0	1 (1)	0	0	0	1 (1)
<i>Typhlocybella</i> sp.	0	0	1 (1)	0	0	1 (1)
<i>Xerophloea</i> sp.	0	1 (1)	0	1 (1)	0	2 (2)
<i>Xestocephalus</i> sp.	0	1 (1)	0	0	0	1 (1)
<i>Xyphon</i> sp.	0	2 (2)	15 (1)	0	0	17 (3)
TOTAL	215	543	535	963	7	2263 (305)

Numbers in parentheses are insects tested for the presence of corn stunt spiroplasma (CSS) and maize bushy stunt phytoplasma (MBSP). \* denotes species that transmit CSS and \*\* denotes species that transmit MBSP (Nault 1980).

2011). We found *D. maidis*, *D. elimatus*, *G. sonora*, and *S. bicolor* on green edge grasses during winter, but none were positive for CSS or MBSP. This result has important implications for edge management because it suggests that although winter grasses maintain potential insect vectors, these vectors are not infected with CSS or MBSP.

An analysis of body size differences (Moya-Raygoza et al. 2007, 2012) suggested that *D. maidis* and *D. elimatus* adults can move both locally and over long distances. *Dalbulus maidis* adults infected with CSS could have migrated to high-elevation sites such as Zapopan from low-elevation sites (less than 1,000 m) elsewhere in Mexico where maize is cultivated (Moya-Raygoza et al. 2007). Also, *D. maidis* infected with CSS can overwinter on volunteer maize. For example, CSS was recovered from *D. maidis* throughout the winter from volunteer maize in California, USA (Summers et al. 2004). No studies have investigated the overwinter presence of MBSP in *D. maidis* or *D. elimatus* in natural conditions. However, it is known that maize plants with MBSP symptoms are found at elevations higher than 1,000 m (Nault 1990). Previous experimental studies have demonstrated that CSS and MBSP are limited to *Zea* species, including maize and its teosinte relatives, whereas other Poaceae such *Tripsacum*, *Coix*, and *Sorghum* species, do not harbor either bacterial pathogen (Nault 1980).

Edge grasses are important, positive habitats because they maintain parasitoids of *D. maidis*. Recently, in the same region of Zapopan, Moya-Raygoza & Becerra-Chiron (2014) found that eggs of *D. maidis* were parasitized by *Oligosita* sp. (Hymenoptera: Trichogrammatidae) and *Anagrus columbi* Perkins (Hymenoptera: Mymaridae) throughout the 2012–2013 winter in perennial grasses habitat. Also in that habitat, dryinids (Hymenoptera) and strepsipterans parasitized the most abun-

dant leafhoppers inhabiting edge grasses in winter, including *S. bicolor*, *G. sonora*, *Exitianus picatus* Gibson, *Amblysellus* sp., and *D. maidis*. In conclusion, the results reported here suggest that leafhoppers that inhabit edge grasses during winter in Zapopan do not harbor CSS or MBSP. On the other hand, past studies have reported that edges are reservoirs of natural enemies of leafhoppers. Future studies examining the presence of CSS and MBSP in leafhoppers that live on edge grasses during different growing seasons and in multiple locations would help confirm this finding.

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## Summary

Leafhoppers (Hemiptera: Cicadellidae) that inhabit edge grasses throughout the winter were tested for the presence of corn stunt spiroplasma (CSS) and maize bushy stunt phytoplasma (MBSP). Leafhoppers were sampled using a sweep net at least once a week between Feb and Jun 2014 in Jalisco, Mexico. In total, 2,263 leafhoppers from 27 different taxa were collected. *Stirellus bicolor* Van Duzee and *Graminella sonora* (Ball) were the most abundant leafhoppers,

with smaller numbers of *Dalbulus maidis* Delong and *D. elimatus* Ball. None of the leafhoppers tested positive for CSS or MBSP, suggesting that these 2 bacterial pathogens do not overwinter in leafhoppers that live on edge grasses. This is an important finding, because several studies have found that crop edges are beneficial reservoirs of insect predators and parasitoids, including those that attack insect vectors of plant pathogens.

Key Words: maize pest; plant pathogen

## Sumario

Las chicharritas (Hemiptera: Cicadellidae) que habitan en los pastos de los bordes del cultivo de maíz, durante el invierno, fueron evaluadas para determinar la presencia del espiroplasma del maíz (CSS) y el fitoplasma del maíz (MBSP). Entre Febrero y Junio del 2014 en Jalisco, Mexico, las chicharritas fueron colectadas al menos una vez por semana, usando una red de golpeo. En total, 2,263 chicharritas de 27 diferentes taxa fueron colectadas. *Stirellus bicolor* Van Duzee y *Graminella sonora* (Ball) fueron las chicharritas más abundantes, se encontraron pocos individuos de *Dalbulus maidis* Delong y *Dalbulus elimatus* Ball. Ninguna de las chicharritas evaluadas, fueron positivas a la presencia de CSS o MBSP, sugiriendo que estas dos bacterias patogénicas al maíz no pasan el invierno dentro de las chicharritas que viven en los pastos de los bordes. Esto es un descubrimiento importante, porque varios estudios han encontrado que los bordes de cultivos son reservorios benéficos para insectos depredadores y parasitoides, incluyendo esos que atacan a insectos vectores de patógenos a plantas.

Palabras Clave: plagas del maíz; patógenos de plantas

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